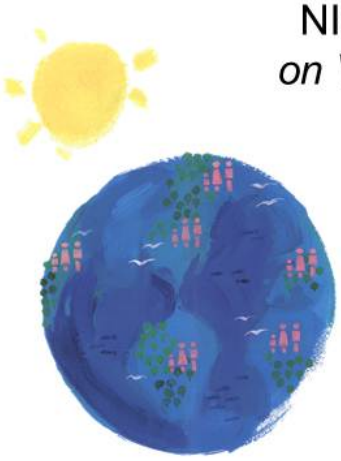
NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Report and Recommendations from the NICEATM-ICCVAM *International Workshop on Vaccine Potency and Safety Testing: State of the Science and Future Directions*



Jodie Kulpa-Eddy, D.V.M., USDA, ICCVAM Chair, ICCVAM BWG Co-Chair

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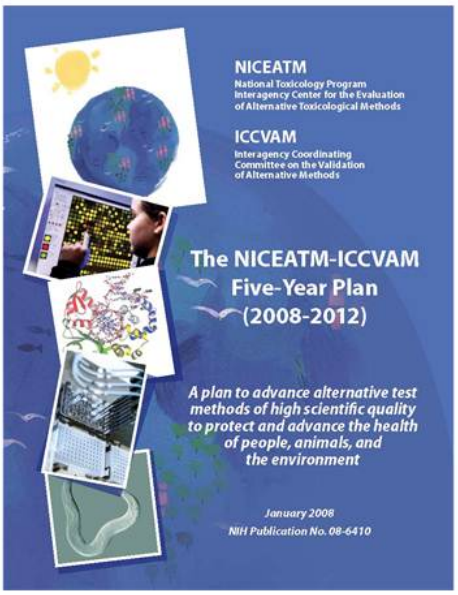
SACATM Meeting
June 17, 2011
Hilton Arlington,
Arlington, VA

NICEATM-ICCVAM Five-Year Plan:2008-2012



- Vaccine Potency and Safety Testing
  - One of ICCVAM's four highest priorities:
    - Large numbers of animals required
    - Involves significant unrelieved pain and distress
    - Multiple agencies involved
- *"NICEATM and ICCVAM will:*
  - *Evaluate alternative test methods and testing strategies for vaccine potency testing*
  - *Facilitate acceptance of adequately validated test methods and humane endpoints found to be sufficiently accurate and reliable."*

Vaccine Potency and Safety Testing Workshop

- NIH, Bethesda, Maryland
- September 14-16, 2010
- Organized by NICEATM-ICCVAM, with ECVAM, JaCVAM, and Health Canada
  - SOT co-sponsorship
- Attended by nearly 200 scientists from 13 countries
- Presentations by:
  - Regulatory authorities
  - Experts from industry
  - Experts from academia
- Poster session

Workshop Goals

1. Review the state of the science of available 3Rs alternative methods for vaccine potency and safety test methods and discuss ways to promote their implementation

2. Identify knowledge and data gaps that need to be addressed to further advance alternative methods for vaccine potency and safety testing

3. Identify and prioritize research, development, and validation efforts needed to address these gaps

Workshop Format

- Opening Plenary Session:
  - Importance of Vaccines to Animal and Human Health
  - National and International Regulatory Programs
- Three Sequential Plenary Sessions:
  - Vaccine Potency Testing Alternatives: Replacements
  - Vaccine Potency Testing Alternatives: Refinement and Reduction
  - Vaccine Safety Testing: 3Rs Alternatives
- Breakout group discussions followed each session
  - Separate groups for human and veterinary vaccines
  - Daily plenary discussion of breakout group recommendations

Vaccine Potency and Safety Testing Workshop: Priorities and Recommendations for Future 3Rs Efforts

Veterinary Vaccines

Jodie Kulpa-Eddy, D.V.M., USDA,
ICCVAM Chair, ICCVAM BWG Co-Chair

Veterinary Vaccine Potency and Safety Assays: Criteria for Prioritization of Future 3Rs Efforts

- Criteria for Prioritization:
  - Vaccine testing requiring large numbers of animals
  - Testing involving significant animal pain and distress
  - Those posing a significant biohazard to workers
  - Those posing a significant animal health hazard: foreign animal diseases
  - Those for which alternatives are undergoing development or validation
  - New vaccines

Veterinary Vaccine Potency and Safety Testing: Recommended Priorities for Future 3Rs Efforts

- Highest Priorities: Potency Testing
  - Rabies
  - *Leptospira sp.* serovars
  - *Clostridium sp.* serovars
  - Erysipelas
  - Fish vaccines
  - Poultry vaccines
  - Foreign animal diseases
  - Non-adjuvanted vaccines
  - Those for which the functional antigen identified/characterized
  - Human & veterinary products for same organism
  - New vaccines
- Highest Priorities: Safety Testing
  - Extraneous agent testing where animals are still used—esp. avian vaccines
  - Inactivation testing for killed vaccines, e.g. rabies
  - Residual toxicity testing
  - Subunit protein or DNA vaccines
  - New vaccines

In Vitro Potency Assays: State of the Science for Veterinary Vaccines

- Many veterinary vaccines do not require the use of animals for batch release potency testing
  - Cell culture techniques
    - Feline Calicivirus
  - Antigen quantification assays
    - Avian Newcastle Disease
    - Canine Leptospiral (non-adjuvanted)
    - Feline Leukemia

Priority Activities for *In Vitro* Veterinary Vaccine Potency Assays

- Focus on identifying protective antigens
- Investigate the relative impact of adjuvants on antigen quantification assays
- Identification of the key epitopes of fish vaccines
- Encouragement of early and frequent interactions with regulators
- Promote increased academic research into test method alternatives

Serological Potency Assays: State of the Science for Veterinary Vaccines

- Veterinary vaccines that utilize Serological Assays to quantify protective antibody in vaccinated animals include:
  - Several *Clostridium sp.*
    - Some still require *in vivo* toxin neutralization test of serum from vaccinated animals
  - Swine Erysipelas Vaccine
  - Canine Leptospira
  - Bovine *Leptospira hardjo*

Priority Activities for Serological Veterinary Vaccine Potency Assays

- Identify and understand the antibodies involved in protective immunity
- Develop and validate assays and reagents to measure antibodies
- Further research on stability of standards/references
- Research into new methods to assess functionality of antibodies or other immune responses
- Rabies: Recommend focused working group of both human and veterinary researchers
  - ***Implementation Activity: Rabies Vaccine Workshop, October 11-13, 2011 (last slide)***
- R&D to convert *in vivo* Toxin Neutralization Tests (TNT) to ELISA or other cell based methods

Veterinary Vaccine Potency Assays with Earlier Humane Endpoints: State of the Science

- All Veterinary vaccines:
  - Euthanasia of moribund animals is current endpoint
  - No requirement for death as the endpoint
- Inactivated Rabies virus vaccine:
  - Paralysis, paresis, convulsions
- Inactivated Swine Erysipelas vaccine:
  - Temperature increase to specific threshold
  - Specific skin rash
- Fowl Pox virus vaccine:
  - Pox lesions, warty eruptions/scabs on combs and wattles

Priority Activities for Using Humane Endpoints in Vaccine Potency Testing

- Identify earlier humane endpoints for vaccines requiring challenge testing
- Collect data and identify clinical endpoints for control groups
- Focus on vaccines for which animals take a longer period of time to develop disease (e.g., *Leptospira sp.*)
- Collect and apply data (clinical observations/measurements) from pre-marketing efficacy tests
- Monitor animals at least twice daily for evidence of humane endpoints or moribund condition
- Guidance on recognition of acceptable clinical signs indicative of moribund condition needs to be communicated and training provided
- Share information between manufacturers and regulators to support change to earlier humane endpoints

Priority Activities for Reduction Alternatives for Veterinary Potency Assays

- Systematic investigation to identify causes of excessive variation and repeat testing
- Investigate ways to reduce or eliminate sources of variation and causes of incomplete test results
- Evaluate feasibility of single dilution assays for challenge and serologic assays
  - e.g., rabies vaccine
- Retrospective review of archival data to determine if control group size can be reduced, but maintain statistical power
- Further investigation into the reduction of animals for secondary stage testing

Veterinary Vaccine Safety Testing Assays: Priority Activities

- Investigate new technologies to characterize vaccine safety
- Investigate cell cultures and PCR for extraneous agent testing
- Investigate cell culture methods for the rabies inactivation test
- Investigate how to develop, validate, and implement safety testing using various tests in a consistency approach

Alternatives for Veterinary Vaccine Testing: Recommendations for Global Progress

- Improve dissemination and accessibility of information on new initiatives, documents, and guidances
- Strive for international harmonization
- Harmonize and achieve global recognition of target antigens
- Establish and maintain universal standard references
  - e.g., OIE, WHO, USDA, EDQM
- Encourage manufacturers to conduct product-specific validation of available alternative methods for their products
- Encourage stakeholder support for research and development of alternative methods

Vaccine Potency and Safety Testing Workshop: Recommendations and Priorities for Future 3Rs Efforts

Human Vaccines

Richard McFarland, M.D., Ph.D., FDA,
ICCVAM BWG Co-Chair

Human Vaccine Potency and Safety Assays: Criteria for Prioritization of Future 3Rs Efforts

- Criteria for prioritization:
  - Number of animals used per test
  - Numbers of lots produced
  - Requirement for *in vivo* challenge test
  - Severity of animal pain and distress
  - Antigens that are well characterized and thus most amenable to developing alternative tests
  - Alternative tests that are in development or already exist, but not yet validated for routine use
  - Combined vaccines
  - Involve nonhuman primates
  - Current tests that are highly variable with significant number of invalid tests

Human Vaccine Potency and Safety Testing: Recommended Priorities for Future 3Rs Efforts

- Highest Priorities: Potency Testing
  - Diphtheria and Tetanus toxoids
  - Pertussis (whole cell and acellular)
  - Rabies
  - Anthrax
  - DTP pentavalent vaccines
  - Inactivated Polio vaccine
  - Combined vaccines
- Highest Priorities: Safety Testing
  - Vero cell assay for Diphtheria
  - Transgenic mouse test for oral polio vaccine (OPV)
  - *In vitro* assay for Tetanus toxoid
  - Histamine (HSA) assay for Pertussis
  - Development of Massively Parallel Sequencing (MPS) and other emerging technologies

In Vitro Potency Assays: State of the Science for Human Vaccines

- To date, *in vitro* antigen quantification tests have been successfully implemented for only a few products
  - Hepatitis A/B vaccines
  - Inactivated polio vaccine
  - Human papillomavirus vaccine
- Pure polysaccharide vaccines have never used animals for batch release
  - Potency of pure polysaccharides vaccines directly related to structure and molecular size
  - Lot release designed to preserve structural epitopes and insure content of high MW polymers

Priority Activities for *In Vitro* Human Vaccine Potency Assays

- Identify key epitopes and their role in protection
- Effects of excipients and adjuvants on *in vitro* systems
- Use of biochemical and physiochemical assays similar to polysaccharide-based vaccines
- Research on the impact of antigen modification due to detoxification and adsorption
- Increase interaction between human and veterinary vaccine regulatory agencies and vaccine manufacturers

Serological Potency Assays: State of the Science for Human Vaccines

- Human vaccines that currently utilize Serological Assays to quantify protective antibody in vaccinated animals include:
  - Tetanus Toxoid Vaccine
  - Diphtheria component in combined Vaccines
  - Whole-cell and acellular Pertussis Vaccine

Priority Activities for Serological Human Vaccine Potency Assays

- Further validation of the Rapid Fluorescent Focus Inhibition Test (RFFIT), an *in vitro* rabies potency test
- Continued efforts towards validation of immunogenicity test for Anthrax vaccine
- Further research necessary to allow broader use of:
  - The Vero cell assay and ELISA for measuring protective response to Diphtheria toxoid
  - The ELISA and ToBI for measuring antibodies to Tetanus toxoid

Human Vaccine Potency Assays and Using Earlier Humane Endpoints

State of the Science

- Inactivated Rabies Virus Vaccine:
  - Paralysis, paresis, convulsions

Priority Activities

- Recognizing clinical signs at the institutional level – training and standardization of classical signs used for humane endpoints is needed
- Routine systematic collection and evaluation of all clinical signs that occur during a challenge test should be done to identify humane endpoints

Priority Activities for Reduction Alternatives for Potency Assays for Human Vaccines

- Require a better understanding of the vaccines that frequently require repeat testing due to invalid results
- Systematic investigation of the sources of variation in current methods
- Re-evaluation of the minimum number of animals required to maintain statistical power
- Use of alternative methods that require fewer animals should be encouraged in those countries that use the full (3 dilutions) test for Diphtheria and Tetanus potency testing
- Promote use of product-specific reference to reduce variability/improve precision

Priority Activities for Safety Testing Alternatives for Human Vaccines

- How to better apply the principles of a consistency approach
- Use of the Vero cell assay to monitor Diphtheria toxin inactivation
- Development of a fully functional *in vitro* assay for Tetanus toxin
- Pending validation, prioritization of the sequence based approach to OPV neurovirulence safety

Alternatives for Human Vaccine Testing: Recommendations for Global Progress

- Allow broader access to information on successfully implemented alternative methods
- Harmonize national requirements on test methods and specifications
- Establish readily available and/or non-proprietary reference standards
- Encourage early and more interaction between manufacturers and regulators
- Recognize the need for harmonization during product development phase
- Encourage sharing of resources including key reagents and assay procedures
- Provide relevant education in bioethics and training in the conduct of 3R assays
- Encourage funding for additional R&D and validation efforts

Acknowledgements: Speakers and Invited Experts

HUMAN VACCINES

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ECVAM Liaison

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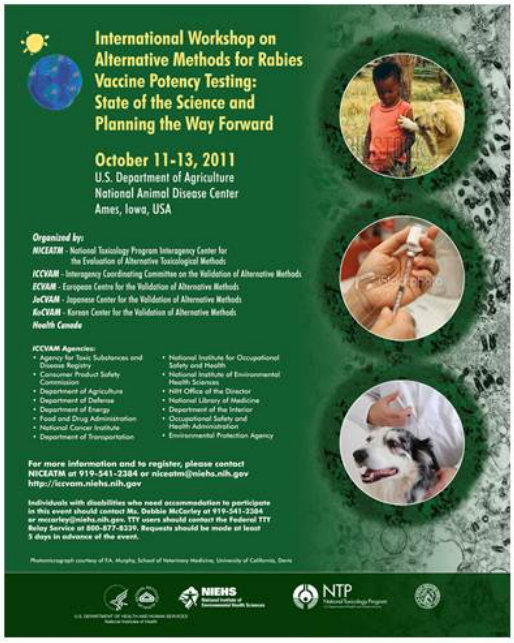
JaCVAM Liaison

Hajime Kojima, Ph.D.

NICEATM-ICCVAM Workshop on Rabies Vaccine Potency Testing

- High priority implementation activity recommended from September 2010 workshop
- This workshop will bring together international scientific experts from government, industry, and academia to:
  - Review the available methods and approaches that reduce, refine, and replace animals used in human and veterinary rabies vaccine potency testing
  - Develop an implementation strategy to achieve global acceptance and use of these alternatives



Save the Date

- October 11-13, 2011
- U.S. Department of Agriculture Center for Veterinary Biologics Ames, IA, USA
- Information and registration available at: http://iccvam.niehs.nih.gov/meetings/RabiesVaccWksp-2011/RabiesVaccWksp.htm

Questions for SACATM (1)

1. Please comment on the overall organization of the workshop in relationship to the stated goals and objectives.
2. The workshop recommended ways to promote the implementation and use of available alternative methods for vaccine potency and safety testing. Do you have additional recommendations?
3. The workshop identified knowledge and data gaps that must be addressed to support the development of alternative methods that can further reduce, refine and replace the use of animals for vaccine potency and safety testing.
   a. Are there additional gaps that should be addressed?
   b. Do you have suggestions for the most appropriate mechanisms and organizations to support the recommended research and development activities?

Questions for SACATM (2)

4. The workshop identified vaccines that should be considered as priorities for future efforts because of the potential for significantly refining, reducing, or replacing animal use for potency and safety testing of these vaccines. Please comment on these priorities, and if there are other high priorities that should be considered.
5. To further promote the implementation of the workshop recommendations, ICCVAM and NICEATM are organizing a workshop in the fall of 2011 to focus specifically on reducing, refining, and replacing animal use for rabies vaccine potency testing.
    a. Please comment on other activities that should be a priority for ICCVAM and NICEATM that would help promote the development, validation, regulatory acceptance, and implementation of alternative methods for vaccine potency and safety testing.